

Article DOI: <https://doi.org/10.3201/eid2310.170307>

# Spread of Chikungunya Virus East/Central/South African Genotype in Northeast Brazil

## Technical Appendix

### Laboratory testing

Viral RNA was extracted from 140  $\mu$ L of serum samples using QIAmp viral RNA kit (QIAGEN, Valencia, CA, USA) and RT-qPCR analyses were performed for DENV serotypes 1–4 (1), ZIKV (2) and CHIKV (3). Of 273 samples tested, 76% ( $n = 208$ ) were CHIKV-RNA<sup>+</sup>, 24% ( $n = 66$ ) were ZIKV-RNA<sup>+</sup> and 13.2% ( $n = 36/273$ ) were co-infected with CHIKV and ZIKV, similar to recent findings from Salvador, Bahia (4).

### Metagenomic sequencing

To identify the cause of the outbreak, each plasma sample was subjected to centrifugation at 15,000 $\times g$  for 10 minutes, filtered through a 0.45  $\mu$ m filter (Merck Millipore, Billerica, MA, USA). The filtrates were treated with a mixture of nuclease enzymes to digest unprotected nucleic acids. Viral nucleic acids were extracted using Maxwell 16 Viral Total Nucleic Acid Purification Kit (Promega, Inc., Madison, WI, USA) and cDNA synthesis was performed with AMV Reverse transcription (Promega, Inc., Madison, WI, USA). A second strand of cDNA synthesis was performed using DNA Polymerase I Large (Klenow) Fragment (Promega, Inc., Madison, WI, USA). Subsequently, a Nextera XT Sample Preparation Kit (Illumina, Inc., San Diego, CA, USA) was used construct a DNA library, with each sample identifiable using dual barcodes. For size selection, we used a Pippin Prep (Sage Science, Inc.) to select a 400bp insert (range 200–600bp). The library was deep-sequenced using the MiSeq Sequencer (Illumina, Inc., San Diego, CA, USA) with 300 bped ends. BLASTx was used to identify viral sequences through their protein sequence similarity to annotated viral proteins in GenBank search, as

previously described (5). Of the 40 samples that were tested, we were able to obtain 23 complete or near-complete genomes (>4000bp; Genbank Accession numbers KY704933 to KY704955).

## Phylogenetic analysis

Publicly available chikungunya virus genome data (>1500bp) was retrieved from Genbank on 17 Feb 2017 using an in-house script. Newly generated genomes (>4000bp) were then appended to the publicly available data and aligned using MAFFT (6). A maximum likelihood phylogeny was constructed for the collated dataset (n = 659) using PhyML with 500 bootstrap replicates (7). The correlation between genetic divergence and collection dates for the ECSA genotype subtree ( $r^2 = 0.41$ , rate =  $8.9 \times 10^{-4}$  substitutions per site per year) was inspected using TemPest (8). Dated phylogenies were constructed using a Bayesian framework implemented in the BEAST software package (9).

To reconstruct date phylogenies, we used a SDR06 nt substitution model with a non-informative Bayesian Skygrid coalescent tree prior (20 grid points) (10) and a strict molecular clock model. Finally, a discrete phylogeographic analysis using an asymmetric substitution model (11) was conducted to investigate the origins and spread across considered locations (n = 3 from Feira de Santana, Bahia, n = 1 from Pernambuco, n = 2 from João Pessoa, Paraíba, n = 2 from Salvador, Bahia, n = 21 from Maceió/Capelas, Alagoas).

## References

1. Lai YL, Chung YK, Tan HC, Yap HF, Yap G, Ooi EE, et al. Cost-effective real-time reverse transcriptase PCR (RT-PCR) to screen for Dengue virus followed by rapid single-tube multiplex RT-PCR for serotyping of the virus. J Clin Microbiol. 2007;45:935–41. [PubMed](#)  
<http://dx.doi.org/10.1128/JCM.01258-06>
2. Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap state, Micronesia, 2007. Emerg Infect Dis. 2008;14:1232–9. [PubMed](#) <http://dx.doi.org/10.3201/eid1408.080287>
3. Lanciotti RS, Kosoy OL, Laven JJ, Panella AJ, Velez JO, Lambert AJ, et al. Chikungunya virus in US travelers returning from India, 2006. Emerg Infect Dis. 2007;13:764–7. [PubMed](#)  
<http://dx.doi.org/10.3201/eid1305.070015>

4. Sardi SI, Somasekar S, Naccache SN, Bandeira AC, Tauro LB, Campos GS, et al. Coinfections of Zika and chikungunya viruses in Bahia, Brazil, identified by metagenomic next-generation sequencing. *J Clin Microbiol.* 2016;54:2348–53. [PubMed](#) <http://dx.doi.org/10.1128/JCM.00877-16>
5. Deng X, Naccache SN, Ng T, Federman S, Li L, Chiu CY, et al. An ensemble strategy that significantly improves de novo assembly of microbial genomes from metagenomic next-generation sequencing data. *Nucleic Acids Res.* 2015;43:e46. [PubMed](#) <http://dx.doi.org/10.1093/nar/gkv002>
6. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol.* 2013;30:772–80. [PubMed](#) <http://dx.doi.org/10.1093/molbev/mst010>
7. Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst Biol.* 2010;59:307–21. [PubMed](#) <http://dx.doi.org/10.1093/sysbio/syq010>
8. Rambaut A, Lam T, de Carvalho L, Pybus OG, Exploring the temporal structure of heterochronous using TemPest, *Virus Evolution* 2:vew007.
9. Drummond AJ, Suchard MA, Xie D, Rambaut A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol Biol Evol.* 2012;29:1969–73. [PubMed](#) <http://dx.doi.org/10.1093/molbev/mss075>
10. Gill MS, Lemey P, Faria NR, Rambaut A, Shapiro B, Suchard MA. Improving Bayesian population dynamics inference: a coalescent-based model for multiple loci. *Mol Biol Evol.* 2013;30:713–24. [PubMed](#) <http://dx.doi.org/10.1093/molbev/mss265>
11. Lemey P, Rambaut A, Drummond AJ, Suchard MA. Bayesian phylogeography finds its roots. *PLOS Comput Biol.* 2009;5:e1000520. [PubMed](#) <http://dx.doi.org/10.1371/journal.pcbi.1000520>

**Technical Appendix Table 1.** Sequencing details of the chikungunya virus isolates reported in this study

| Accession number | Isolate name | No. reads mapped | Mean depth coverage | Genome coverage (%)* |
|------------------|--------------|------------------|---------------------|----------------------|
| KY704933         | 6AL          | 19,267           | 175.79              | 79.02                |
| KY704934         | 14           | 5,221            | 54.18               | 62.27                |
| KY704935         | 17AL         | 2,847            | 44.05               | 73.63                |
| KY704936         | 19           | 11,517           | 53.39               | 61.72                |
| KY704937         | 151          | 3,064            | 47.40               | 48.03                |
| KY704938         | 164          | 1,010            | 16.90               | 67.16                |
| KY704939         | 166          | 36,801           | 467.31              | 94.97                |
| KY704940         | 167          | 1,992            | 32.16               | 72.51                |
| KY704941         | 168          | 796              | 16.79               | 52.21                |
| KY704942         | 172          | 18,577           | 249.79              | 95.53                |
| KY704943         | 175          | 8,561            | 82.14               | 78.84                |
| KY704944         | 178          | 1,004            | 21.48               | 60.91                |

| Accession number | Isolate name | No. reads mapped | Mean depth coverage | Genome coverage (%)* |
|------------------|--------------|------------------|---------------------|----------------------|
| KY704945         | 182          | 74,785           | 328.01              | 51.82                |
| KY704946         | 188          | 6,927            | 77.66               | 66.64                |
| KY704947         | 190          | 9,650            | 146.31              | 84.09                |
| KY704948         | 191          | 13,041           | 62.36               | 56.36                |
| KY704949         | 194          | 44,321           | 335.58              | 59.84                |
| KY704950         | 195          | 16,140           | 168.99              | 67.76                |
| KY704951         | 197          | 650              | 16.38               | 58.19                |
| KY704952         | 241          | 1,192,684        | 2,050.39            | 93.76                |
| KY704953         | 243          | 3,342            | 50.44               | 74.80                |
| KY704954         | 19AP         | 15,832           | 151.30              | 100.00               |
| KY704955         | 35AP         | 11,206           | 111.64              | 100.00               |

\*Relative to reference genome sequence KU940225.

**Technical Appendix Table 2.** Clinical information of sequenced chikungunya virus isolates\*

| Isolate | CT   | Sex, age (y) | Date     | Location                  | Clinical symptoms  |
|---------|------|--------------|----------|---------------------------|--|
| 6AL     | 24.9 | F, 44        | 30-03-16 | Santa Lúcia, Maceió, AL   | Fever, arthralgia  |
| 14      | 21.4 | M, 30        | 30-03-16 | Poço, Maceió, AL          | Fever, arthralgia, muscle pain, headache                 |
| 17AL    | 21.7 | F, 50        | 01-04-16 | Centro, Maceió, AL        | Fever, muscle pain, nausea                               |
| 19      | 19.5 | F, 31        | 01-04-16 | Santos, Maceió, AL        | Fever, arthralgia, myalgia, fatigue, exanthema           |
| 151     | 35.8 | M, 64        | 15-04-16 | Tab. do Pinto, Maceió, AL | Fever, arthralgia, edema                                 |
| 164     | 33.9 | F, 25        | 19-04-16 | Clima Bom, Maceió, AL     | Fever, arthralgia, headache                              |
| 166     | 29.5 | M, 34        | 17-04-16 | Poço, Maceió, AL          | Fever, arthralgia, headache                              |
| 167     | 28.2 | M, 30        | 14-04-16 | Vergel, Maceió, AL        | Fever, arthralgia, muscle pain                           |
| 168     | 36.9 | F, 43        | 14-04-16 | Vergel, Maceió, AL        | Fever, arthralgia, headache                              |
| 172     | 18.2 | M, 18        | 16-04-16 | FernãoVelho, Maceió, AL   | Fever, arthralgia, headache                              |
| 175     | 19.5 | F, 28        | 19-04-16 | Poço, Maceió, AL          | Arthralgia, exanthema                                    |
| 178     | 20.2 | M, 41        | 19-04-16 | Centro, Maceió, AL        | Fever, pruritus  |
| 182     | 19.3 | F, 17        | 14-04-16 | Santa Amélia, Maceió, AL  | Fever, arthralgia, maculopapular rash                    |
| 188     | 25.8 | F, 30        | 17-04-16 | Centro, Capela, AL        | Fever, arthralgia, headache, nausea                      |
| 190     | 36.0 | F, 61        | 15-04-16 | Mangabeiras, Maceió, AL   | Fever, arthralgia, myalgia, headache, retro-orbital pain |
| 191     | 28.8 | F, 36        | 19-04-16 | Farol, Maceió, AL         | Fever, headache, myalgia, arthralgia, leukopenia         |
| 194     | 23.3 | F, 60        | 07-04-16 | Pajuçara, Maceió, AL      | Fever, arthralgia, myalgia, fatigue                      |
| 195     | 17.6 | F, 39        | 07-04-16 | Poço, Maceió, AL          | Fever, arthralgia, myalgia, fatigue                      |
| 197     | 21.8 | F, 21        | 13-04-16 | Farol, Maceió, AL         | Fever, myalgia, arthralgia                               |
| 241     | 19.8 | M, 11        | 07-04-16 | Centro, Maceió, AL        | Fever, headache, maculopapular rash, vomiting            |
| 243     | 26.2 | F, 41        | 09-04-16 | Sta. Amélia, Maceió, AL   | Muscle pain, skin rash                                   |
| 19AP    | 16.5 | F, 20        | 20-06-16 | Centro, João Pessoa, PB   | Vomiting, Fever, Headache, maculopapular rash            |
| 35AP    | 26.4 | F, 32        | 17-06-16 | Bayeux, João Pessoa, PB   | Muscle pain, maculopapular rash                          |

\*Location indicates neighborhood, municipality and federal state (AL = Alagoas, PB = Paraíba). Date of sample collection is shown as dd-mm-yy. CT = qPCR-CT value.